Gene Delivery in Tissue Engineering: A Photopolymer Platform to Coencapsulate Cells and Plasmid DNA

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Purpose. Toward the ultimate goal of developing an engineered tissue capable of mimicking complex natural healing processes, we have designed a photopolymer platform that enables simultaneous encapsulation of cells and plasmid DNA in degradable hydrogels. Photopolymerization enables spatial and temporal control of gel formation under physiological conditions, but the presence of photoinitiator radicals poses challenges for DNA photoencapsulation.

Methods. The effects of photoinitiating conditions (ultraviolet light and photoinitiator radicals) on plasmid DNA were studied. Protection methods were identified. Plasmid DNA was photoencapsulated in photocrosslinked hydrogels, and the quantity and quality of the released DNA were assessed. Plasmid DNA was simultaneously entrapped (coencapsulated) with cells in hydrogels to assess *in situ* transfection.

Results. Experiments showed that in the absence of other species, plasmid DNA was sensitive to photoinitiator radicals, but the addition of transfection agents and/or antioxidants greatly reduced DNA damage by radicals. Encapsulated plasmid DNA was released from degradable, photocrosslinked hydrogels in active forms (supercoiled and relaxed plasmids) with an overall ~60% recovery. Released DNA was capable of transfecting both plated and encapsulated cells. Encapsulated cells expressed the encoded gene of the coencapsulated plasmid as the polymer degraded.

Conclusions. This photopolymerization platform allows for the creation of engineered tissues with enhanced control of cell behavior through the spatially and temporally controlled release of plasmid DNA.

KEY WORDS: controlled release; DNA; photopolymer; tissue engineering.

INTRODUCTION

Tissue engineers are continually exploring new strategies to modulate *in vivo* healing processes or enable *ex vivo* generation of neotissues. When the body builds new tissues, as during wound healing and morphogenesis, numerous biochemical signals are utilized in a temporally and spatially regulated manner. Sophisticated tissue engineering strategies are evolving in an effort to mimic the critical features of natural tissue regeneration. One way in which tissue engineers are better mimicking natural tissue regeneration is by delivering proteins from a polymer scaffold (1–3). Proteins play an active role in tissue regeneration; however, it is experimentally difficult to maintain protein stability during scaffold processing and polymer degradation because of their fragile three-dimensional structure. An alternative to proteins is to deliver plasmid DNA that encodes therapeutic proteins.

Plasmid DNA offers several advantages over delivering the therapeutic protein directly (4–6). In addition to DNA's greater stability in a wide range of environments, methods developed for delivering plasmid DNA should apply to plasmids encoding many different proteins, and from a manufacturing perspective, plasmid DNA is considerably easier to produce and purify than recombinant proteins. Most importantly, delivering plasmid DNA allows an additional means of control of the therapeutic protein concentration. DNA sequences that control gene expression in response to external stimulants or in response to internal biochemical signals can be incorporated into the plasmid to enhance control of the foreign protein expression.

Because DNA delivery circumvents some of the challenges associated with protein delivery and provides additional means of controlling cell behavior, the tissue engineering community is interested in designing scaffolds that incorporate DNA and identifying approaches to sustain its release for the transfection of local or delivered cells. DNA released from a polymer matrix has the potential to transfect local cells and to utilize the cell's own machinery to synthesize therapeutic proteins at appropriate levels. Despite these advantages, there are many barriers to efficient delivery of genes to cells and tissues (7–9). Transfection agents continue to be developed to overcome these difficulties facing efficient cellular delivery of DNA both *in vitro* and *in vivo*. Many of the *in vivo* barriers to gene delivery may be avoided by delivering the plasmid DNA locally, thereby avoiding systemic interactions. In addition to enhanced, localized transfection, the delivery of DNA from a controlled release device provides continuous transfections, which should prolong the expression of the foreign gene, inducing a longer-lasting therapeutic effect and reducing the need for repeated treatment.

Researchers have successfully released DNA from both natural and synthetic polymer matrices. Alginate, chitosan, and collagen are natural polymers that form crosslinked networks via ionic and/or chemical crosslinking and are desirable for DNA encapsulation because of their mild gelation conditions. Aggarwal *et al.* (10) showed that plasmid DNA released from alginate microspheres injected into the mouths of rats was capable of transfecting cells in the intestines, spleen, and liver. Aral *et al.* (11) observed higher levels of transfection by plasmid DNA released from chitosan microspheres injected into muscle tissue than for injection of the naked plasmid. Bonadio (6) explored the use of collagen matrices for the delivery of DNA to migrating, wound-healing cells. As secreted enzymes cleaved the matrix, DNA was released from these collagen-based gene-activated matrices, enabling transfection of migrating cells. Although the scaffold properties and degradation rate of these natural polymer matrices can be controlled to some extent by altering the extent of crosslink-

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ABBREVIATIONS: PLGA, poly(lactic-co-glycolic acid); SEAP, secreted alkaline phosphatase; GFP, green fluorescent protein; UV, ultraviolet; PEGPLADM, dimethacrylated PLA-b-PEG-b-PLA copolymer; PBS, phosphate-buffered saline; PEG, polyethylene glycol; PLA, poly(lactic acid); LA, lactic acid units; r, relaxed; l, linear; sc, supercoiled.

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ing (12), synthetic polymers allow even greater control of the scaffold macroscopic properties and degradation rate, which are critical for many tissue engineering applications.

One of the most commonly investigated synthetic polymers for gene delivery and tissue engineering is poly(lactic-co-glycolic acid) (PLGA) (5,13–18). Evidence of transfection has been detected for DNA released from both PLGA microspheres (13–16) and nanospheres (17,18) despite an observed decline in plasmid integrity during release (13). DNA damage is thought to be caused by the organic solvents and high shear forces involved in the formation of the PLGA particles and/or the low pH environment within the degrading microspheres and nanospheres. Shea *et al.* (5) incorporated and released plasmid DNA from porous PLGA matrices formed via a milder gas foaming procedure. The sustained and localized delivery of naked plasmid DNA (encoding platelet-derived growth factor) enhanced blood vessel formation in the fibrous capsule surrounding the subcutaneous implant and led to the transfection of about 1000 times more cells than plasmid injected without polymer. However, *in vitro* release studies showed that the percentage of DNA released in its supercoiled conformation decreased from ~43% to 0% over 28 days. Thus, despite the ubiquitous use of PLGA for drug delivery and tissue engineering applications, these data suggest that the PLGA polymer chemistry and/or its degradation products negatively impact DNA quality over time.

As an alternative synthetic polymer, hydrogels, and especially photopolymerized hydrogels, are seeing increased application in tissue engineering as cell and protein delivery vehicles (1,19–22). However, little research has focused on DNA encapsulation and delivery from such synthetic hydrogels. Recently, Nakayama *et al.* (22) reported the release of adenovirus-associated DNA from vascular stents coated with a photopolymerized hydrogel. Although the authors showed evidence of transfection of endogenous cells by the photoencapsulated DNA, they did not address the effects of photoencapsulation on DNA quality.

We propose that a photopolymerized hydrogel platform would be an ideal environment for simultaneously encapsulating DNA and cells while allowing controlled DNA delivery to the cells and ultimately enabling or accelerating tissue regeneration. In this work, we demonstrate for the first time the ability to photoencapsulate DNA with cells in degradable hydrogels. Using this dual delivery system, we show that DNA conformational stability is sustained within photopolymerized hydrogels and that the delivered DNA is capable of transfecting encapsulated cells at a rate dictated by the gel degradation kinetics. This photopolymerization platform will provide many benefits for designing DNA delivery matrices for tissue engineering applications. In particular, gels are formed under physiological conditions, and thus, the monomer may be injected and polymerized *in situ*. Furthermore, photoinitiated polymerization allows spatial and temporal control of gelation, which permits matrices to be created with patterned depots of DNA. Such patterning allows spatially controlled transfection or the creation of regions having different degradation rates, allowing temporally controlled transfection. In addition, plasmids that encode several different tissue-inducing proteins could be located in these various regions to better mimic the complex natural healing processes.

MATERIALS AND METHODS

Materials

DNA plasmids that encode secreted alkaline phosphatase (SEAP, Clontech, Palo Alto, CA) and green fluorescent protein (GFP, Aldevron, Fargo, ND) were purchased from commercial vendors. Dimethacrylated poly(lactic acid)-b-poly(ethylene glycol)-b-poly(lactic acid) triblock copolymers (PEG-PLA-DM monomers) were synthesized according to previously reported procedures (23,24). Monomers were synthesized with the same PEG core molecule (4000 g/mol, Fluka, Switzerland) but with different numbers of lactic acid units ranging from 5 LA (lactic acid units) to 17 LA per molecule (see general structure, Fig. 1). Methacrylation efficiency ranged from 90% to 100% as indicated by ^{1}H NMR. Photopolymerizations were initiated with 4-(2-hydroxyethoxy)phenyl-(2-propyl)ketone (I-2959, Ciba-Geigy, Basel, Switzerland) and a 365-nm lamp (Cole-Parmer, Vernon Hills, IL) at an intensity of 5 mW/cm^2.

DNA Protection Studies

To study the effects of ultraviolet (UV) light on DNA, solutions of DNA were prepared in deionized water at a concentration of 10 μg/ml. These solutions (100 μl) were exposed to 365-nm light at 5 mW/cm^2 or to 254-nm light at 1 mW/cm^2 (UVP, Upland, CA). UV damage was examined by agarose gel electrophoresis. DNA samples were loaded at 20–50 ng per lane on a 0.8 wt% agarose gel (high melting temperature agarose, Fisher, Pittsburgh, PA). Electrophoresis was conducted at 100 V for 45 minutes. Gel photographs were analyzed with Kodak 1D software (Kodak, Rochester, NY).

To study the effects of photoinitiator radicals on DNA, 10 μg/ml DNA was added to 0.05 wt% I-2959 initiator solutions prepared with deionized water. DNA was complexed to

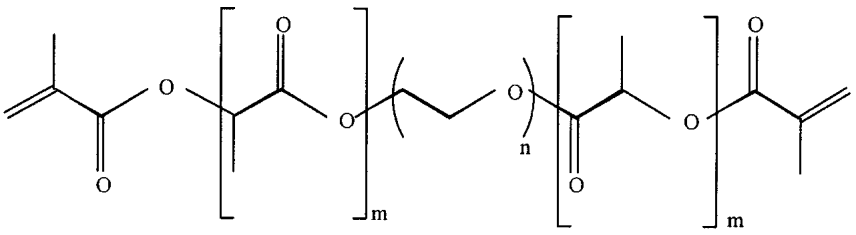

Fig. 1. Chemical structure of the PEGPLADM monomer. Square brackets define the PLA block where m indicates the number of lactic acid units per block. For all monomers discussed in this work, the PEG core has a molecular weight of 4000 or n≈91.

a variety of transfection agents before the solutions were exposed to UV light. Transfection agents included PEI (polyethylenimine, 25 kDa, Sigma, St. Louis, MO), Lipofectamine™ (Invitrogen, San Diego, CA), FuGENE™ (Roche, Basel, Switzerland), protamine sulfate (Sigma, St. Louis, MO), and liposomes of DOTAP (1,2-dioleoyl-3-trimethylammonium-propane, Avanti Polar Lipids, Alabaster, AL) with cholesterol (Sigma, St. Louis, MO) prepared as described elsewhere (25). Complexes were formed for 20 min with commonly used ratios: PEI, 3 μl of 10 mM PEI/μg DNA (N/P = 10); Lipofectamine, 3 μl/μg DNA; FuGENE, 3 μl/μg DNA; protamine sulfate, 0.6 mg/μg DNA; DOTAP/Chol liposomes, 0.8 mg DOTAP/μg DNA. After exposure, PEI–DNA and protamine sulfate–DNA complexes were decomplexed by the addition of 300 mg/ml sodium tripolyphosphate (Acros, Fairlawn, NJ); all lipid-DNA complexes were decomplexed by the addition of 1 M sodium dodecyl sulfate (Aldrich, Milwaukee, WI). The protective effects of L-ascorbic acid (Sigma, St. Louis, MO) were studied by adding 0.01–0.5 g/L of ascorbic acid (vitamin C) to the initiator solutions before exposing them to UV light. The DNA/initiator solutions (250 μl) were then transferred to 1.7 ml clear polypropylene microcentrifuge tubes and exposed to 365-nm light at 5 mW/cm^2 for 10 min. DNA was quantified using the PicoGreen assay (Molecular Probes, Eugene, OR), which detects only double-stranded DNA.

DNA Photoencapsulation

Ten percent by weight PEGPLADM monomer and 0.05 wt% I-2959 initiator were dissolved in deionized water, and plasmid DNA was added to the monomer/initiator solution at loadings of 2–6 μg of DNA/mg of monomer. It should be noted that this range of DNA loading does not significantly affect the polymerization or DNA release (data not shown). Where noted, plasmids were complexed with transfection agents before being mixed into the monomer solution. The monomer/DNA mixture was exposed to 5 mW/cm^2 of 365-nm light for 10 min to produce the degradable hydrogels. The PEGPLADM hydrogels were approximately 8 mm in diameter and 3.2 mm in depth.

Gel Degradation and DNA Release Studies

Polymerized gels were degraded in 2–5 ml phosphate-buffered saline (PBS, pH 7.4) at 37°C on an orbital shaker. Buffer was removed periodically, and the DNA concentration was determined by the PicoGreen assay. Parallel gels were degraded that contained no DNA to account for the effects of the polymer degradation products on the PicoGreen assay. Fresh buffer was added to the gels, which were then returned to the shaker.

Quality of Released DNA

Quality of the released DNA was examined with both agarose gel electrophoresis and cell transfections. Agarose gel electrophoresis was conducted as described above. Cell transfections were conducted with NIH 3T3 cells (ATCC, Manassas, VA). Cells were seeded in 12-well plates and allowed to reach ~75% confluence. DNA samples were complexed with Lipofectamine at 3 μl Lipofectamine/μg DNA in the sample. After the DNA complexes had been given 20 min to form, the plated cells were washed with PBS, 1 ml Opti-MEM (Gibco, Gaithersburg, MD) medium was added to each well, and 2 μg of DNA in complexed form was added to each well. After 5 h, the transfection medium was removed, cells were rinsed with PBS, and 1 ml of complete medium (DMEM with 10% calf serum, Invitrogen, Carlsbad, CA) was added to each well. After 2 days, evidence of transfection was measured. To detect transfection by plasmids that encode SEAP, medium samples were removed and assayed for SEAP activity using a SEAP assay kit (Clontech, Palo Alto, CA) and a Wallac Victor 2 fluorometer (Perkin-Elmer, Wellesley, MA).

Coencapsulation of Cells with DNA

DNA was first complexed with Lipofectamine for 15–20 min in deionized water, while PEGPLADM monomer (4000MW PEG core, 4.5 LA per PLA block, 100% methacrylated) was dissolved in the solution of DNA complexes to provide a ratio of 4.5 μg of DNA/mg monomer. The mixture was then frozen overnight at –80°C. The frozen DNA/monomer solutions were lyophilized (Freezone 4.5, LabConco, Kansas City, MO) for 24 h. The freeze-dried DNA/monomer solutions were rehydrated with deionized water to make a 10 wt% monomer solution. Primary chondrocytes (cartilage-forming cells) were isolated from a calf knee as described previously (26). The isolated chondrocytes were centrifuged at 200 × *g* for 10 min. The cell pellet was then resuspended with the rehydrated DNA/monomer solution at a concentration of ~50 million cells/ml. This cell suspension was then transferred in 40-μl aliquots to the barrel of 1-ml syringes and exposed to 365-nm light at 5 mW/cm^2 for 10 min. The resulting chondrocyte–polymer–DNA gels were incubated in 2 ml of chondrocyte medium [prepared as described previously (26)] on a rotating platform in a 37°C CO_2 incubator. Medium was replaced every 2–3 days. To detect transfection of the encapsulated chondrocytes by the entrapped plasmids encoding GFP, photographs of the cells were taken every 2–3 days using a Nikon TE 300 fluorescent microscope fitted with a FITC filter cube (excitation 480 nm, emission 535nm; Chroma Corporation, McHenry, IL).

RESULTS AND DISCUSSION

Photoinitiation in the Presence of DNA

Before photoencapsulating DNA in a hydrogel, we assessed the stability of DNA under photoinitiation conditions commonly used for cell encapsulation and tissue engineering applications [i.e., a cytocompatible photoinitiator (27) activated by long-wave ultraviolet (UV) light]. Figure 2 shows the damage induced by two different wavelengths of UV light on plasmid DNA as detected by agarose gel electrophoresis. The supercoiled form (bottom band) is often considered the preferred form of DNA for transfection, but the relaxed form, which can occur with mild environmental changes such as freezing and thawing, appears to be capable of transfecting cells (28). When significantly damaged, plasmid DNA may adopt a linear form indicating that the circular molecule has been cleaved by harsh environmental conditions or DNAses. In the linear form, the plasmid is no longer active and cannot produce the encoded protein.

As Fig. 2 shows, exposing DNA to 254-nm light resulted

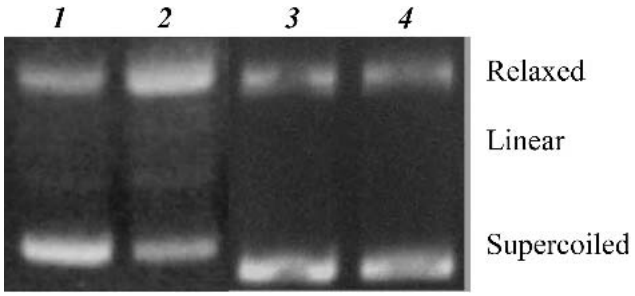

Fig. 2. Agarose gel electrophoresis of aqueous solutions of plasmid DNA exposed to UV light in two separate experiments. Lane 1 shows unexposed DNA, Lane 2 shows DNA after exposure to 254 nm light (1 mW/cm^2 for 10 minutes). Lane 3 shows unexposed DNA, Lane 4 shows DNA after exposure to 365 nm light (5 mW/ cm^2 for 10 minutes).

in a 50% decrease in the supercoiled form (lane 2), yet there was also a 30% loss in detectable DNA (indicating denaturation). Others have demonstrated similar DNA damage in response to short-wave UV light (29). In contrast, when DNA was exposed to 365-nm light, there was no DNA denaturation and only a slight (~5%) decrease in supercoiling (lane 4). Thus, 365-nm light was not deemed to be detrimental to DNA at the cytocompatible intensities and exposure times characteristic of our photopolymerizations, and all subsequent experiments were conducted with 365-nm light.

Because the initiating light is not damaging to DNA, the primary concern with photoencapsulating DNA is the presence of highly reactive radicals that initiate the chain polymerization. Radicals are formed when a photoinitiator molecule is cleaved on exposure to light. The rate of radical generation depends on the efficiency of the initiator radicals to form propagating radicals, the molar extinction coefficient of the initiator at the initiating wavelength, the incident light intensity, and the initiator concentration (27). The rate of polymerization can be controlled by manipulating the radical concentration via any of these factors affecting the rate of radical generation. Facile control of the polymerization rate is particularly important in encapsulating cells, where the polymerization must be completed on a physiologically acceptable time scale.

Researchers have shown that radicals are highly damaging to DNA (30), and our experiments confirm that photoinitiated radicals damage naked plasmid DNA. Only ~2% of the DNA is detectable after exposure to typical photoinitiating conditions (Table I). Clearly, the reactive radicals generated by the photoinitiator significantly damage DNA in the absence of other species, and thus, methods were developed to protect DNA from radical attack. Addition of a radical scavenger, ascorbic acid (vitamin C), at 0.1 g/L reduced DNA damage about 20-fold. Vitamin C reacts with and inactivates initiator radicals generated by the photoinitiating system. Unfortunately, at higher concentrations ($\geq$ 0.2 g/L), vitamin C also suppressed polymerization and prevented macroscopic hydrogel formation. With lower concentrations of vitamin C present during polymerization ($\leq$ 0.1 g/L), a gel was formed; however, the gel degraded in slightly less time. The decreased degradation time may result from a lesser extent of reaction during polymerization or from shorter kinetic chains that typically result from the addition of an inhibitor or chain transfer agent. Both of these effects have been reported by others (31) exploring the chain polymerization of crosslinked networks. Ideally, the radical scavenger would be localized to the vicinity of the DNA to inhibit radicals in the immediate environment of the DNA without inhibiting the overall polymerization.

An alternative to radical scavenging is to complex the DNA to a transfection agent. By changing the DNA conformation into a more compact form (32) and providing a molecular barrier around the DNA, transfection agents leave fewer DNA sites open for radical attack. In our experiments, transfection agents protected about 20–60% of the DNA from radicals (Table I); however, the addition of vitamin C (0.1 g/L) to DNA complexed to any of several transfection agents increased the amount of DNA protected by the transfection agents by ~50–200%. Thus, in the absence of other species in solution, plasmid DNA is highly susceptible to radical attack. Transfection agents and vitamin C greatly reduce damage of the DNA by radicals, and the incorporation of such protectants during photoencapsulation may be beneficial for obtaining high DNA recovery from the photopolymerized gels.

Releasing Photoencapsulated DNA

Figure 3 shows naked DNA release profiles from degrading PEG-based hydrogels (chemical structure shown in Fig. 1). The data are normalized to the total amount of DNA released from the gels. The DNA release rate is dictated by

Table I. Percent of DNA Detected Relative to the Initial DNA Concentration (10 μg/ml) after Exposure to Photoinitiator Radicals Generated by a 0.05 wt% I-2959 Solution, and 10 Minutes of Exposure to 365-nm light at 5 mW/cm^2

Transfection agent	% Detectable DNA	
	No vitamin C	With 0.15 g/L vitamin C
None	2.4 ± 0.8%	39 ± 29%
Lipofectamine	21 ± 2.3%	55 ± 14%
FuGENE	28 ± 7.3%	n/a
Protamine sulfate	28 ± 8.5%	83 ± 16%
DOTAP: Cholesterol	52 ± 22%	n/a
DOTAP: Cholesterol: Protamine Sulfate	52 ± 9.7%	75 ± 13%
Polyethylenimine	60 ± 9.3%	88 ± 5.2%

Note: Samples containing transfection agents were decomplexed prior to assay with sodium tripolyphosphate or sodium dodecyl sulfate (average ± 1 standard deviation of 4–10 samples per condition).

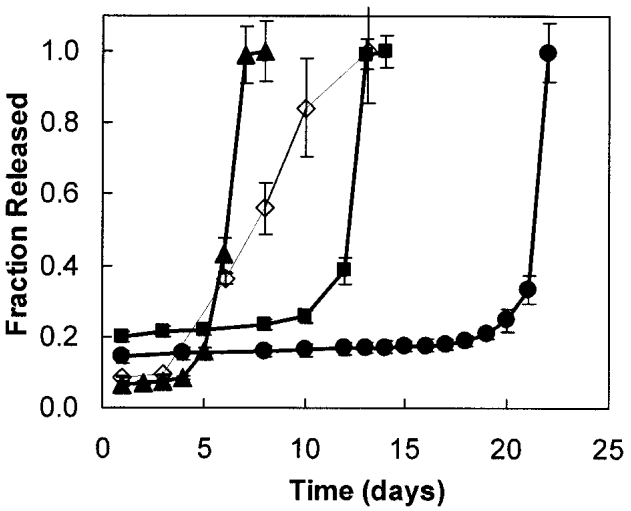

Fig. 3. Fractional naked DNA release as a function of time from PEGPLADM hydrogels with varying monomer chemistry. Raw release data were normalized to the total amount of DNA released from each gel to yield fraction released. Bold symbols denote homopolymers composed of a core PEG (4000 g/mol) with degradable PLA blocks of (▲) 8.5 lactic acid units (LA)/PLA block, (■) 5 LA/PLA block, and (●) 2.5 LA/PLA block. The open symbols (◇) show the DNA release from a copolymer composed of 7wt% 8.5 LA/PLA block, 2wt% 5 LA/PLA block, and 1wt% 2.5 LA/PLA block.

the polymer degradation kinetics, and for homopolymers (polymers formed from a single monomer), rapid DNA release is observed as the gel is completely eroded. To control the DNA release profile, the monomer chemistry and/or monomer combination is varied. Figure 3 illustrates the effects of the size of the degradable PLA blocks in the monomer on DNA release and the DNA release from a copolymer formed from several different monomers. With a homopolymer containing a higher molecular weight PLA block [8.5 lactic acid units (LA)/block], the gel degrades in one-third the time it takes for a gel formed from monomer containing 2.5 LA/block. Each lactic acid repeat unit in the PLA block contains an ester linkage susceptible to hydrolytic cleavage at physiological pH, and thus, the more lactic acid repeat units in the monomer, the faster the crosslinks are cleaved, and thus the gel degrades in less time. Interestingly, when DNA is released from a polymer composed of three monomers containing various molecular weight PLA blocks, the release profile is substantially different. Rather than a burst in DNA release, the DNA is released steadily after a short initial lag. These data show that minor changes in monomer chemistry and monomer combinations can have significant effects on gel degradation and DNA release, which implies the ability to precisely tailor the gel for a given application. Even more slowly degrading gels may be obtained by further decreasing the size of the PLA block or by changing the ester chemistry to poly(caprolactone), which is more hydrolytically stable than PLA (data not shown).

Although the rate of DNA release is important to a gene delivery system, the total amount of DNA delivered and the activity of the released DNA are critical to the success of the controlled release system. Unexpectedly, when DNA was photoencapsulated in hydrogels with no protective agent present, about 50% of the loaded DNA was recovered. It is surprising that unprotected DNA has such a high recovery because only about 2% of unprotected DNA was recovered in radical exposure studies conducted with the same concentration of photoinitiator in the absence of monomer. To determine if transfection agents and radical scavengers might further improve DNA recovery after photoencapsulation in these hydrogels, DNA was complexed to protamine sulfate and/or vitamin C was added to the monomer/initiator mixture before the encapsulation of the DNA. Interestingly, there was not a statistically significant increase in DNA recovery with any of the protective additives (vitamin C, 47 ± 2% recovery; protamine sulfate, 61 ± 5% recovery; both protamine sulfate and vitamin C, 69 ± 12% recovery). Although these particular agents were insufficient to protect significantly more DNA during encapsulation, other transfection agents and localization of vitamin C around the DNA should improve DNA recovery from the photopolymerized gels.

Quality of Photoencapsulated and Released DNA

Figure 4 shows that the photoencapsulated DNA was released from the PEG-based hydrogel primarily in the relaxed and supercoiled forms with minimal occurrence of the inactive, linear form. The first lane in each image shows unencapsulated DNA in a solution of degraded polymer, and the second lane shows the DNA released late in the degradation (during the burst portion of the release). Complexing the DNA with protamine sulfate before photoencapsulation had little effect on the quality of the released DNA compared to unprotected DNA. Image analysis revealed that both cases gave a ratio of relaxed (r): linear (l): supercoiled (sc) of ~80%:15%:5%. Use of vitamin C, on the other hand, more effectively preserved the supercoiled form of the plasmid DNA (r:l:sc of 66%:10%:24%). The combined protection by protamine sulfate and vitamin C prevented the damage leading to the linear form of the DNA and greatly reduced the loss of the supercoiled form, showing r:l:sc of 57%:0%:43%., which was similar to the unencapsulated DNA, r:l:sc of 39%:0%:61%. Thus, although these protective additives did not increase the quantity of released DNA, they did improve the quality. Such high retention of the supercoiled form throughout polymer degradation (~70% of the original supercoiled form present at the end of polymer degradation when pro-

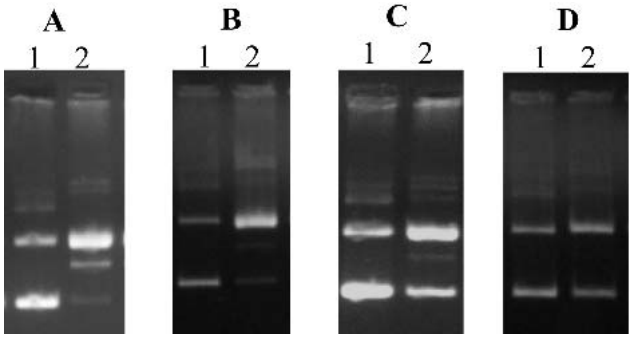

Fig. 4. Agarose gel electrophoresis of released, photoencapsulated DNA from PEGPLADM gels with 5 LA/PLA block. Lane 1 shows un-encapsulated DNA; lane 2 shows released DNA. (A) naked DNA, (B) protamine-complexed DNA, (C) naked DNA with vitamin C present during photoencapsulation, (D) protamine-complexed DNA with vitamin C present during photoencapsulation.

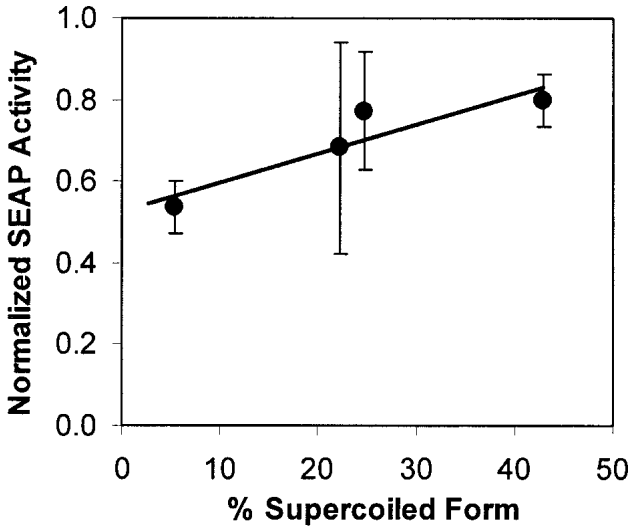

Fig. 5. Transfection efficiency as a function of the percent supercoiled plasmid DNA released from photopolymerized hydrogels. Transfection efficiency is calculated as the secreted alkaline phosphatase (SEAP) activity detected in cells transfected with released DNA divided by the SEAP activity in cells transfected with unencapsulated DNA spiked into the appropriate degraded polymer solution (average ± 1 standard deviation, n = 3).

tected with vitamin C and protamine sulfate) is a significant improvement over PLGA based systems that show no supercoiled form present prior to complete degradation (5). Even with no protection, ~10% of the original supercoiled form was present when the photocrosslinked hydrogel completely degraded. Thus, these highly water-swollen PEG-based hydrogels seem to provide a more suitable environment for maintaining DNA conformation than the more hydrophobic PLGA scaffolds. In addition, the concentration of acidic degradation products from the PEG-based hydrogel is considerably lower than from PLGA matrices, which may contribute to the improved DNA stability in the photopolymerized hydrogel over time.

To further evaluate the quality of photoencapsulated and released DNA, we tested the ability of the plasmid to produce the encoded protein (secreted alkaline phosphatase) in cell transfections. Four samples taken from the delayed burst portion of the release were tested for their ability to transfect cells (Fig. 5). The three DNA samples released in the presence of protective additives (vitamin C, protamine sulfate, and both additives) were compared to an unprotected DNA sample. Each sample contained different amounts of the supercoiled, relaxed, and linear forms, and as expected, the samples with the highest amount of the supercoiled form gave the highest level of transfection (up to 80% of the signal for unencapsulated DNA control[1]). This trend of increasing transfection efficiency with the supercoiled form of the DNA reiterates the importance of releasing plasmid DNA in the supercoiled form, and the high levels of transfection clearly demonstrate the ability of these photocrosslinked gels to release active DNA even at late stages in the gel degradation.

Transfection of Encapsulated Cells: Application to Tissue Engineering

For cartilage tissue engineering, methods have previously been developed for the photoencapsulation of chondrocytes (cartilage-forming cells) (26,27) demonstrating excellent cell viability and an ability to generate cartilaginous tissue *in vitro*. The addition of DNA to this system provides an opportunity to alter cell behavior and enhance tissue formation.

In Fig. 6 we show that photoencapsulated chondrocytes can be successfully transfected by coencapsulated plasmid DNA encoding green fluorescent protein (GFP). The encapsulated chondrocytes expressed increasing GFP as the gel eroded and the encapsulated Lipofectamine™-complexed DNA was delivered to the cells. Starting around day 5, GFP signal was visible above the background noise, and from about day 10 onward, the majority of cells expressed GFP. For comparison, the temporal expression of GFP by cells transfected before encapsulation is shown. Interestingly, in gels with coencapsulated DNA and chondrocytes, expression of the transfected gene was more widespread but at lower levels than observed for chondrocytes transfected before encapsulation. Later time points are not shown because, unfortunately, these transfected, encapsulated cells were unable to produce sufficient extracellular matrix to hold the construct together before the polymer degraded. With these chondrocyte–polymer constructs, the rate of polymer degradation must be carefully timed to coincide with the formation of extracellular matrix in order to maintain construct integrity. Experiments are under way identifying appropriate polymer compositions for use with transfected cells.

Our ultimate goal is to control tissue formation in these polymer–cell constructs, and to date we have demonstrated the ability to simultaneously coencapsulate cells and DNA in a single photopolymer gel and that the delivered DNA transfects these encapsulated cells. Furthermore, this transfection of encapsulated cells by coencapsulated DNA is evidence of the suitability of photocrosslinked hydrogels for the codelivery of cells and DNA. Because the transfection of the encapsulated cells by the entrapped DNA is dictated by the gel degradation kinetics, transfection can be controlled temporally and spatially in this photopolymer system by using combinations of slowly and rapidly degrading polymers or by localization of the plasmid DNA in regions of the gel.

CONCLUSIONS

We have shown that photoencapsulated DNA can be released from photocrosslinked and degradable hydrogels in an active, supercoiled form. The addition of radical scavengers (such as vitamin C) and complexing the DNA with transfection agents (such as protamine sulfate) do not appear to improve the overall recovery of DNA but instead preserve the integrity of the plasmid DNA during photoencapsulation. The photoencapsulated, released DNA is capable of transfecting both plated and encapsulated cells.

This photopolymerization approach is unique in its abil-

[1]An important note about these cell transfections with released DNA is that the polymer degradation products greatly interfere with transfection. Cell transfections with nonencapsulated DNA spiked into a degraded polymer solution result in ~5% of the protein signal obtained from nonencapsulated DNA in water. These cell transfections were conducted merely as a test of the quality of the released DNA and do not reflect the methods by which cells are to be transfected from these polymers.

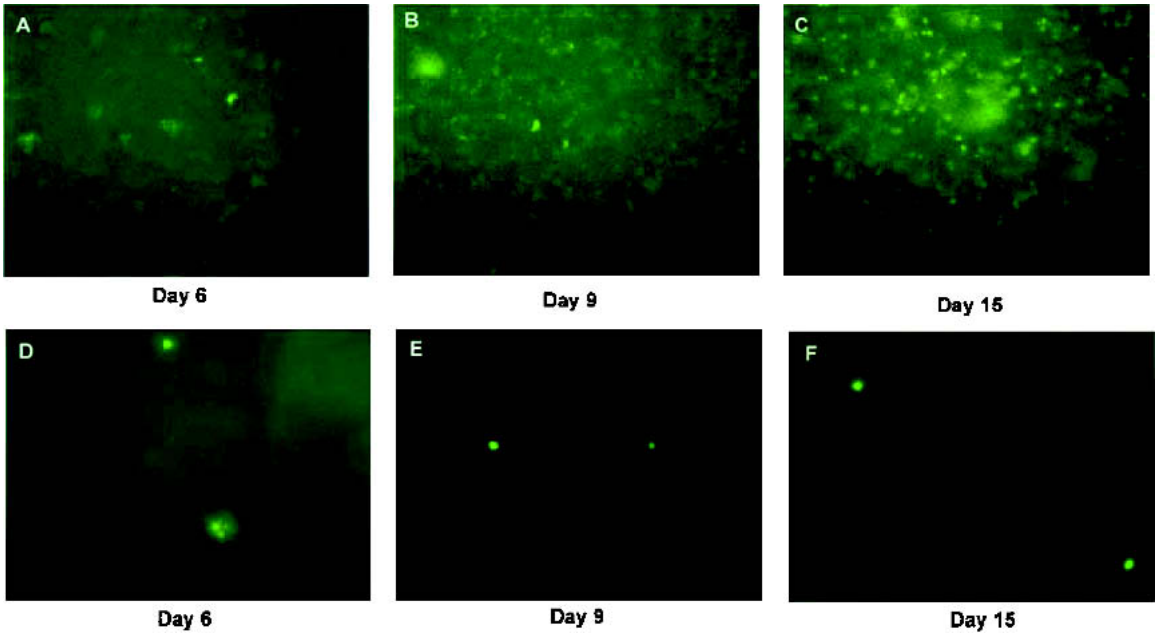

Fig. 6. Fluorescence micrographs of GFP expression over time by encapsulated chondrocytes. (A–C) Lipofectamine-complexed DNA was released from PEGPLADM hydrogels (2.5 LA/PLA block) to encapsulated chondrocytes. (D–F) Chondrocytes were transfected prior to encapsulation in PEGPLADM hydrogels.

ity to simultaneously photoencapsulate cells and DNA and thus provides a new dual delivery platform for tissue engineering applications in which both cells and inductive factors need to be delivered. A single-pronged approach of simply delivering polymer, cells, or inductive factors does not appear to be sufficient to mimic the multifaceted nature of natural wound healing processes. The stimulation of cells with bioactive factors (such as plasmid DNA) in a spatially and temporally regulated manner while providing a tissue-inductive polymer scaffold will be key to developing a more native-like tissue. Photopolymerization allows the facile development of such complicated systems with options for complex polymer structures, homogeneous cell encapsulation, spatial and/or temporal release of tissue-inductive factors, and also *in situ* polymerization.

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